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Application for Patent

אני, (שם המבקש, מענו ולגבי גוף מאוגד - מקום התאגדותו)
I (Name and address of applicant, and in case of body corporate-place of incorporation)

ידע חברה למחקר ופיתוח בע"מ,
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בעל אמצאה מכח Assignment העברה שמסה הוא
Owner, by virtue of of an invention the title of which is

(בעברית) כימרה של IL6RIL6 לטיפול במחלות הגורמות דמיילינציה
(Hebrew)

(באנגלית) IL6RIL6 Chimera for the Treatment of Demyelinating Diseases
(English)

מבקש בזאת כי ינתן לי עליה פטנט
hereby apply for a patent to be granted to me in respect thereof.

• בקשת חלוקה — Application of Division	• בקשת פטנט מוסף — Application for Patent Addition	• דרישה רין קדימה Priority Claim		
מבקשת פטנט from Application	• לבקשה/לפטנט to Patent/Appl.	מספר/סימן Number/Mark	תאריך Date	מדינת האגוד Convention Country
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היום 21 בחודש יוני שנת 1999
This 21 of June of the year 1999

לשימוש הלשכה
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IL6RIL6 CHIMERA FOR THE TREATMENT OF DEMYELINATING DISEASES

כימרה של IL6RIL6 לטיפול במחלות הגורמות דמיילינציה

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Y/99-67

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FIELD OF THE INVENTION

The present invention is in the field of nerve myelination and generation of cells which produce the myelin sheath. In particular it relates to the use of IL6RIL6 chimeras for producing pharmaceutical compositions for the treatment of demyelinating diseases and the enhancement of nerve regeneration.

BACKGROUND OF THE INVENTION

Nerve myelination is an essential process in the formation and function of the central nervous system (CNS) and peripheral nervous system (PNS) compartments. The myelin sheath around axons is necessary for the proper conduction of electric impulses along nerves. Loss of myelin occurs in a number of diseases, among which are Multiple Sclerosis (MS) affecting the CNS, Guillain-Barre Syndrome, CIDP and others (see 'Abramsky and Ovadia, 1997; Trojaborg, 1998,Hartung et al, 1998). While of various etiologies, such as infectious pathogens or auto-immune attacks, demyelinating diseases all cause loss of neurological functions and may lead to paralysis and death. While present therapeutical agents reduce inflammatory attacks in MS and retards disease progression, there is a need to develop therapies that could lead to remyelination and recovery of neurological functions (Abramsky and Ovadia, 1997, Pohlau e al, 1998).

The synthesis of myelin is a function of specialized glial cells: the oligodendrocytes in the CNS and the myelinating Schwann cells in the PNS. These two cell types in their fully differentiated state may be called myelinating cells. Myelin is a lipid membrane structure containing a number of different proteins. Myelin basic proteins (MBP) represent the major components (30%) of CNS and also of PNS myelin proteins. Expression of the MBP and other genes encoding the various myelin proteins (e.g. Po, PMP-22, MAG in PNS, PLP, MOG in CNS), is turned on during the terminal differentiation of oligodendrocytes and myelinating Schwann cells. The origin of these cells is in the embryonal neural crest (Fraser, 1991) from which they migrate, and undergo a differentiation that proceeds in a number of

steps. Schwann cell (SC) development appears to involve three main steps: 1) the generation of precursors (pSC) from migrating cells; 2) the proliferation and transition to embryonic SC (eSC) expressing the S100 protein; 3) the postnatal terminal differentiation of part of the eSC population into myelinating SC that express MBP and other myelin proteins (Kioussi and Gruss, 1996). The cells migrating from the neural crest give rise not only to pSC but also to sensory and sympathic neurons, to smooth muscle cells and to cells which reach the skin and hair follicles and become pigmented melanocytes. The fate of the neural crest cells is affected by various inducing factors: differentiation to glial cells, to neurons and to muscles is promoted by Neuregulins such as glial growth factor (GGF), by BMP2/4 and by TGF-β respectively (Anderson, 1997). The differentiation to melanocytes may be promoted by growth factors such as bFGF or PDGF or SDF (Stocker et al, 1991; Anderson, 1997).

The ultimate differentiation of Schwann and oligodendrocyte progenitors into actively myelinating cells and myelination itself seems to depend on signals generated by the interaction between neuronal axons and the glial cells (Lemke and Chao, 1988; Trapp et al, 1988). When axon-Schwann cell contact is interrupted, as after nerve damage, the cells reverse to a non-myelinating state and expression of myelin protein genes is lost (Jessen and Mirsky, 1991). To be able to stimulate myelination or remyelination, after neural diseases or trauma, it would be extremely important to identify factors that are able to induce the synthesis of myelin.

Neuregulins such as GGF, which act on embryonic Schwann cell precursors, are also survival, growth and maturation factors for postnatal oligodendrocytes and Schwann cells in damaged nerves, and GGF is one of the mitogenic factors provided by axonal contact (Topliko et al, 1996). Recombinant hGGF2 could enhance remyelination upon prolonged administration in a murine model for Multiple Sclerosis (Cannella et al, 1998) or in crushed peripheral nerve (Chen et al, 1998). Another cytokine that is induced in Schwann cells by axonal contact is the Ciliary neurotrophic factor CNTF (Lee et al, 1995). CNTF, as well as leukemia inhibitory factor (LIF), was shown to promote survival of oligodendrocytes from optic nerve cultured in vitro with bFGF or PDGF, and to increase the number of MBP expressing oligodendro- cytes in these cultures (Mayer et al, 1994). However, when added to glial precursor cells, CNTF and LIF appear rather to favor astrocyte differentiation and induce expression of the astrocyte GFAP marker, while on oligodendrocytes it would have mainly a survival action with little effect on the level of MBP gene expression (Kahn and De

Vellis, 1994, Bonni et al, 1997). Nevertheless, combinations of CNTF with brain-derived neurotrophic factor BNDF improve recovery of an injured peripheral sciatic nerve (Ho et al, 1998).

CNTF and LIF are cytokines acting through a common receptor system which comprises the LIF receptor (LIFR) and the gp130 chain, the latter being also part of the Interleukin-6 (IL-6) receptor complex (Ip et al, 1992). CNTF and LIF are, therefore, part of the IL-6 family of cytokines. In the case of CNTF and LIF, signal transduction operates through dimerization of LIFR with gp130, whereas in the case of IL-6 the signal is generated by the dimerization of two gp130 chains (Murakami et al, 1993). In order to bind gp130, IL-6 makes a complex with an IL-6 Receptor chain, which exists on certain cells as a gp80 transmembrane protein but whose soluble form can also function as an IL-6 agonist when provided from outside the cell (Taga et al, 1989, Novick et al, 1992). By fusing the entire coding regions of the cDNAs encoding the soluble IL-6 receptor (sIL-6R) and IL-6, a recombinant IL6RIL6 chimera can be produced in CHO cells (Chebath et al, 1997, WO99/02552). This IL6RIL6 chimera has enhanced IL-6-type biological activities and it binds with a much higher efficiency to the gp130 chain in vitro than does the mixture of IL-6 with sIL-6R (Kollet et al, 1999).

A review of the effects of IL-6 on cells of the central and peripheral nervous system indicates that the cytokine may have protective effects on neuronal cells as well as participate in inflammatory neuro-degenerative processes (Gadient and Otten, 1997, Mendel et al, 1998). On glial cells, CNTF and LIF were much more active than IL-6 to stimulate astrocyte differentiation and there was no effect on myelin protein producing cells (Kahn and De Vellis, 1994). In transgenic mice expressing higher levels of both IL-6 and soluble IL-6R (sIL6-R), an accelerated nerve regeneration was observed following injury of the hypoglossal nerve as shown by retrograde labeling of the hypoglossal nuclei in the brain (Hirota et al, 1996). In that work, the addition of IL-6 and sIL-6R to cultures of dorsal root ganglia (DRG) cells showed increased neurite extension in neurons, but no effect on myelinating cells was reported.

In the light of the data presented above, CNTF, LIF or a mixture of IL-6 and sIL-6R have not been shown to induce the terminal differentiation of glial cells into myelinating cells. However, as outlined above, stimulation of myelinating cells differentiation would be of great benefit for patients suffering from demyelinating or neurodegenerative diseases.

Citation of any document herein is not intended as an admission that such document is pertinent prior art, or considered material to the patentability of any claim of the present application. Any statement as to content or a date of any document is based on the information available to applicants at the time of filing and does not constitute an admission as to the correctness of such statement.

SUMMARY OF THE INVENTION

It is an aim of the present invention to provide a means to stimulate the differentiation of progenitors or differentiated glial cells into myelinating cells. The basis of the invention is in the use of the recombinant IL6RIL6 chimera protein from CHO cells, which has a markedly higher affinity for gp130 than does the mixture of IL-6 and sIL-6R.

It is also an aim of the present invention to show that the IL6RIL6 chimera increases the number of Schwann cells developing in dorsal root ganglia (DRG) cultures. Furthermore, it is an aim of the present invention to show that IL6RIL6 induces the differentiation of these cells to the point where they wrap around axons and produce myelin basic protein.

It is another aim of the present invention to show that the IL6RIL6 chimera induces the transcription of the myelin basic protein (MBP) genes in a system of transdifferentiation in which cells with melanocytic phenotype are converted into a Schwann, myelinating phenotype, as illustrated by the effect of IL6RIL6 on a murine melanoma.

A molecular mechanism is proposed by which the IL6RIL6 induces and represses specific transcription factors that bring about the induction of the MBP gene differentiation into myelinating phenotype.

Thus, the present invention provides use of IL6RIL6 chimera to produce a pharmaceutical composition for treating traumatic nerve degeneration, demyelinating diseases of the CNS or PNS and/or neurodegenerative diseases.

In particular, the invention provides for the use of IL6RIL6 chimeras in the treatment of multiple sclerosis (MS).

The invention also provides pharmaceutical composition comprising IL6RIL6 chimera, optionally together with one or more pharmaceutically acceptable excipients, for treating traumatic nerve degeneration, demyelinating diseases of the CNS or PNS and/or neurodegenerative diseases.

A preferred use for the pharmaceutical compositions of the present invention is the treatment of MS.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 shows the increase in MBP RNA in cultures of Schwann cells treated by IL6RIL6 chimera or by forskolin (FSK) or left untreated (NT).

Fig. 2 shows that IL6RIL6 strongly induces MBP mRNAs (A) and decreases Pax-3 mRNA (B) over time in the F10.9 cells. NT designate non treated cells.

DETAILED DESCRIPTION OF THE INVENTION

We have surprisingly found that addition of IL6IL6R recombinant protein to culture of dorsal root ganglia cells or of melanoma cells stimulates the differentiation of those cells into myelinating cells.

The present invention concerns the use of the "IL6RIL6 chimera" (also called "IL6RIL6"), which is a recombinant glycoprotein obtained fusing the entire coding sequence of the naturally-occurring soluble IL-6 Receptor δ-Val to the entire coding sequence of mature naturally-occurring IL-6, both from human origin. The IL6RIL6 chimera is produced in genetically engineered CHO cells as described in WO 99/02552.

More particularly, the present invention concerns the use of IL6RIL6 chimera to stimulate the differentiation of progenitor or differentiated glial cells into myelinating cells. As demonstrated herein the IL6RIL6-induced myelinating cells differentiation process involves both, activation of genes required for the formation of the myelin sheath around neuronal axons, as well as repression of a gene required for the maintenance of non myelating phenotypes .

In accordance with the present invention it has been observed that the addition of IL6RIL6 chimera to cultures of embryonic dorsal root ganglia (eDRG) cells, isolated from mice embryos at days 14-15 of gestation, has a profound effect on the development of the Schwann cell precursors present in the DRG. After 2-5 days in culture, there is a marked increase in the number of the embryonic Schwann cells, a marked phenotypic change in

these cells which start to wrap their membrane around the DRG axons, and an induction of MBP.

Furthermore it has also been found according to the present invention that the addition of IL6RIL6 chimera to cultures of the B16/F10.9 murine melanoma cell line, induces the expression of the MBP gene within 6-12 hours. Other genes which encode proteins of the myelin, such as the CNPase gene are induced whereas expression of genes which are involved in melanogenesis (formation of melanin pigments) such as tyrosinase, are strongly repressed. The F10.9 cells treated by IL6RIL6 also undergo a marked morphological change, and acquire a Schwann-like phenotype. The phenotypic changes and the induction of specific myelin genes support the hypothesis that IL6RIL6 causes a transdifferentiation of the cells from a melanocytic to myelinating state. Since in the embryo, cells migrating from the neural crest can give rise to either melanocytes or myelinating Schwann cells and oligodendrocytes, it is suggested that IL6RIL6 can influence the fate of the cells and promote the formation of myelinating cells.

Moreover, it is shown in the accordance with the present invention that IL6RIL6 acts by down-regulating the homeobox gene Pax-3, a gene expressed in embryonic neural crest cells before they differentiate into myelinating Schwann cells (Kioussi and Gruss, 1996). Pax-3 is known to repress MBP gene. Therefore, Pax-3 repression appears to be a key event in the final maturation of the myelinating cells. Hence IL6RIL6 acts on a key differentiation switch (i.e. pax-3 respression).

Pax-3 is a transactivator of the micropthalmia associated transcription factor MITF, which in turn induces and maintains the expression of the tyrosinase and other genes responsible for the melanocytic phenotype. The discovery of the rapid repression of Pax-3 by IL6RIL6 can therefore explain the molecular events which promote the myelinating activity of the neural crest derived cells. After nerve injury, myelinated axons undergo demyelination as part of the Wallerian degeneration. During that process, Schwann cells turn down the expression of MBP gene and other related myelin protein genes. Concomitantely there is an upregulation of Pax-3 and GFAP which denotes a reversion from myelinating SC to non-myelinating and proliferating SC (Kioussi and Gruss, 1996). In accordance with the present invention, the IL6RIL6 chimera appears to be a potent cytokine to revert the process of Wallerian nerve degeneration by repressing Pax-3 and inducing the SC to resume their myelinating activities. The same considerations would apply to brain demyelinating diseases

since, like in traumas, the neurodegeneration in these diseases is spurred by a demyelination process driven by macrophages and other inflammatory cells.

IL6RIL6 can be injected to mice in which an autoimmune demyelination has been induced by immunization with MBP as a model system for chronic relapsing multiple sclerosis (Cannella et al, 1998). The capacity of IL6RIL6 to induce myelin protein genes and differentiation of myelinating glial cells can be observed in vivo, using this pharmaceutical paradigm.

The definition of "pharmaceutically acceptable" is meant to encompass any carrier, which does not interfere with effectiveness of the biological activity of the active ingredient and that is not toxic to the host to which it is administered. For example, for parenteral administration, IL6RIL6 chimera may be formulated in a unit dosage form for injection in vehicles such as saline, dextrose solution, serum albumin and Ringer's solution.

The IL6RIL6 chimera can be administered to an individual in a variety of ways. The routes of administration include intradermal, transdermal (e.g. in slow release formulations), intramuscular, intraperitoneal, intravenous, subcutaneous, oral, epidural, topical, and intranasal routes. Any other therapeutically efficacious route of administration can be used, for example absorption through epithelial or endothelial tissues or by gene therapy wherein a DNA molecule encoding the IL6RIL6 chimera is administered to the patient (e.g. via a vector) which causes the IL6RIL6 chimera to be expressed and secreted *in vivo*. In addition the IL6RIL6 chimera can be administered together with other components of biologically active agents such as pharmaceutically acceptable surfactants, excipients, carriers, diluents and vehicles.

For parenteral (e.g. intravenous, subcutaneous, intramuscular) administration, IL6RIL6 chimera can be formulated as a solution, suspension, emulsion or lyophilized powder in association with a pharmaceutically acceptable parenteral vehicle (e.g. water, saline, dextrose solution) and additives that maintain isotonicity (e.g. mannitol) or chemical stability (e.g. preservatives and buffers). The formulation is sterilized by commonly used techniques.

An "effective amount" refers to an amount of the active ingredients that is sufficient to affect the course and the severity of the diseases described above, leading to the reduction or remission of such pathology. The effective amount will depend on the route of administration and the condition of the patient.

The dosage administered, as single or multiple doses, to an individual will vary depending upon a variety of factor, including IL6RIL6 chimera pharmacokinetic properties, the route of administration, patient conditions and characteristics (sex, age, body weight, health, size), extent of symptoms, concurrent treatments, frequency of treatment and the effect desired. Adjustment and manipulation of established dosage ranges are well within the ability of those skilled, as well as *in vitro* and *in vivo* methods of determining the remyelination of the nerves.

While the invention will be described in conjunction with specific embodiment thereof, it will be understood that it is capable of further modifications. This application is intended to cover any variations, uses or adaptations of the invention following, in general, the principles of the invention and including such departures from the present disclosure as come within known or customary practice within the art to which the invention pertains and as may be applied to the essential features hereinbefore set forth as follows in the scope of the appended claims.

All references cited herein, including journals articles or abstracts, published or unpublished patent applications, issue or foreign patents, or any other references, are entirely incorporated by references herein, including all data, tables, figures, and text presented in the cited references. Additionally, the entire contents of the references cited within the references cited herein are also entirely incorporated by references.

The present invention will now be described in more detail in the following non-limiting Examples and the accompanying drawings.

EXAMPLES

Example 1: Effect Of IL6RIL6 on Myelination in Cultures of Embryonic Dorsal Root Ganglia

In the spinal cord, the dorsal root comprises essentially sensory neurons, which form synapses in the dorsal root ganglia (DRG). During the mouse embryogenesis (at day e14-e15), DRGs are a convenient source of neurons and of embyonic Schwann cells which have not yet differentiated into myelinating SC. Procedures to obtain explants of DRGs for in vitro cultures are described by Li (1998). The cultures were performed on glass coverslips placed in the wells of Costar plates, in medium F12/DMEM (Gibco). The coverslips were coated either with collagen or with poly-D-lysine, with essentially similar results. Cultures

were either done in medium without growth factor or cytokine additions or in medium supplemented with nerve growth factor (NGF, 40 ng/ml), or in medium supplemented with IL6RIL6 chimeric recombinant proteins (3 µg/ml). The cultures were examined daily by light microscopy with an Olympus inverted microscope linked to a video-camera imaging system (Leica LIDA system). Part of the coverslips were fixed in paraformaldehyde and MBP proteins were labeled with monoclonal primary antibodies to myelin basic proteins and fluorescein-conjugated secondary antibody. Neuronal cell bodies and axons were stained with antibodies to neurofilament protein. Some of the coverslips were examined by scanning electron microscopy (EM).

After 2 to 5 days, the DRG explants cultured without addition showed that the cells growing out of the explant were either polygonal or oval-shaped. However, when NGF was added, the oval shaped cells developed long axonal projections which formed a thin network stained by antibodies to neurofilaments. Some of the axons were long and bifurcated, but no Schwann cells were observed along the axons. In contrast, cultures with IL6RIL6 showed not only neuronal cells with axons stained for neurofilament, but also Schwann cells appearing as flat cells that had long bipolar extensions with end ramifications. These extensions were not stained for neurofilament proteins. By scanning EM, these Schwann cells were clearly observed along the axonal projections with membrane rufflings starting to wrap around the axon.

Staining with anti-MBP showed positively stained Schwann cells in the IL6RIL6-treated cultures, in particular in arrays of cells which were aligned one after the other. On the other hand, little MBP-specific fluorescence was seen in the NGF-treated cultures without IL6RIL6.

Similar results were observed in DRG cultures from e15 rat embryos.

Schwann cells derived from mouse sciatic nerve were also cultured in vitro with IL6RIL6 1.4 µg/ml and the level of MBP RNA transcript was measured. In comparison, the same cultures were treated with forskolin 20 µM, a chemical which artificially increases the cyclic AMP levels in the cells and is known to induce MBP (Lemke and Chao, 1988). The results showed that IL6RIL6 was as efficient as forskolin to induce MBP gene expression and more efficient to maintain MBP RNA levels after 3 days of culture (Fig. 1).

Example 2: Induction of MPB gene by IL6RIL6 in cultures of melanoma B16-F10.9 cells

The embryonic origin of skin melanocytes, which produce the melanin pigments, and of Schwann cells is from common precursor cells migrating out of the neural crest in e8 mouse embryos. Melanomas are malignant tumors developing in the skin from melanocytes, and are therefore also derived from neural crest ancestors. The B16 cell line is derived from a spontaneous melanoma of Balb/c mice, and the F10.9 clone was isolated from B16 for its highly malignant metastatic phenotype. The F10.9 cells, as other B16 cells, produce black eumelanin pigment and are rich in tyrosinase, the first enzyme of the melanogenic pathway (Bertolotto et al, 1996).

F10.9 cells were seeded in 96-well microplates at 30,000 cells/well and cultured for 3 days in DMEM medium with 10% FCS, without or with IL6RIL6 at concentrations of 0.3-1 µg/ ml. Total cell RNA was extracted and analyzed by Northern blots with cDNA probes for MBP. The MBP mRNAs were induced very strongly at 48 by IL6RIL6 in the F10.9 cells (Fig. 2A). A time course study showed that the increase in MBP RNA started at 12 hours following the addition of the IL6RIL6 chimera to the cell cultures.

The IL6RIL6 chimera induces not only MBP gene expression but also the cyclic 2'3' AMP phosphodiesterase or CNPase, which is another component of the myelin and a marker for differentiated Schwann cells. The cells also developed prolonged extensions at opposite poles of the cell body, and aligned in long arrays as typical of Schwann cells in cultures.

Surprisingly, IL6RIL6 switched the phenotype of the F10.9 cells from melanin producing cells to myelin producing Schwann cells. The tyrosinase enzymatic activity and the production of melanin was completely lost at 48 hours after addition of the IL6RIL6 chimera to the cells.

MITF is a transcription factor that activates the tyrosinase gene (Bertolotto et al. 1996). The treatment of F10.9 cells by IL6RIL6 strongly repressed the MITF gene expression. MITF itself is transactivated by the homeotic transcription factor Pax-3 (Watanabe et al. 1998) and measurements of Pax-3 mRNA in the IL6RIL6 treated F10.9 cells showed that Pax-3 expression decreases starting from 6 hours and up to 48 hours (Fig. 2B). Pax-3 is known to repress the MBP gene (Kioussi and Gruss, 1996). The effect of the IL6RIL6 chimera can, therefore, be ascribed to a gene regulation effect on the Pax-3 homeobox gene, which is expressed during embryonic Schwann cell development prior to

myelination, and has to be repressed for myelination to occur. Moreover, in degenerating demyelinating nerves, Pax-3 is re-expressed in the Schwann cells of when these cells stop to produce MBP. It is, therefore, of great importance that IL6RIL6 can both repress Pax-3 and cause the differentiation of Schwann cells into myelinating cells by inducing myelin protein genes.

Example 3: Injections of IL6RIL6 in a murine model of chronic relapsing multiple sclerosis.

Mice of the SJL/J strain develop experimental autoimmune encephalomyelitis (EAE) following immunization with 0.4 mg bovine MBP in incomplete Freund's adjuvant containing 60 µg Mycobacterium tuberculosis H37Ra (Difco). The disease can be passively transferred to syngeneic recipient by intravenous injection of 30 million lymphnode cells taken 10 days after immunization. The clinical signs of paralysis appear after a week to 10 days. An acute phase of disease is followed later by remissions and relapses. IL6RIL6 is injected to these mice intraperitoneally or subcutaneously at doses of 1, 3 and 5 µg per mice (body weight about 25 g). The injections are given 4 times per week for at least 3 weeks, starting at either day 3 or day 7 after the passive transfer. The clinical score of the animals is followed and graded as: 1) loss of tail rigidity; 2) hindlimb weakness; 3) limb paralysis on one side; 4) limb paralysis on both sides; 5) lethality. The brain and spinal cord of animals is examined by light microscopy following staining of myelin by luxol fast blue. The effect of IL6RIL6 on reduction in clinical grade and reduction of demyelination of white brain matter can be ascertained.

Having now fully described the invention, it will be appreciated by those skilled in the art that the same can be performed within a wide range of equivalent parameters, concentrations and conditions without departing from the spirit and scope of the invention and without undue experimentation.

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CLAIMS

1. Use of IL6RIL6 chimera to produce a pharmaceutical composition for treating traumatic nerve degeneration, demyelinating diseases of the CNS or PNS and/or neurodegenerative diseases.

2. The use of claim 1 wherein the demyelinating disease is multiple sclerosis (MS).

3. Pharmaceutical composition comprising IL6RIL6 chimera, optionally together with one or more pharmaceutically acceptable excipients, for treating traumatic nerve degeneration, demyelinating diseases of the CNS or PNS and/or neurodegenerative diseases.

4. The pharmaceutical composition of claim 3 wherein the demyelinating disease is MS.

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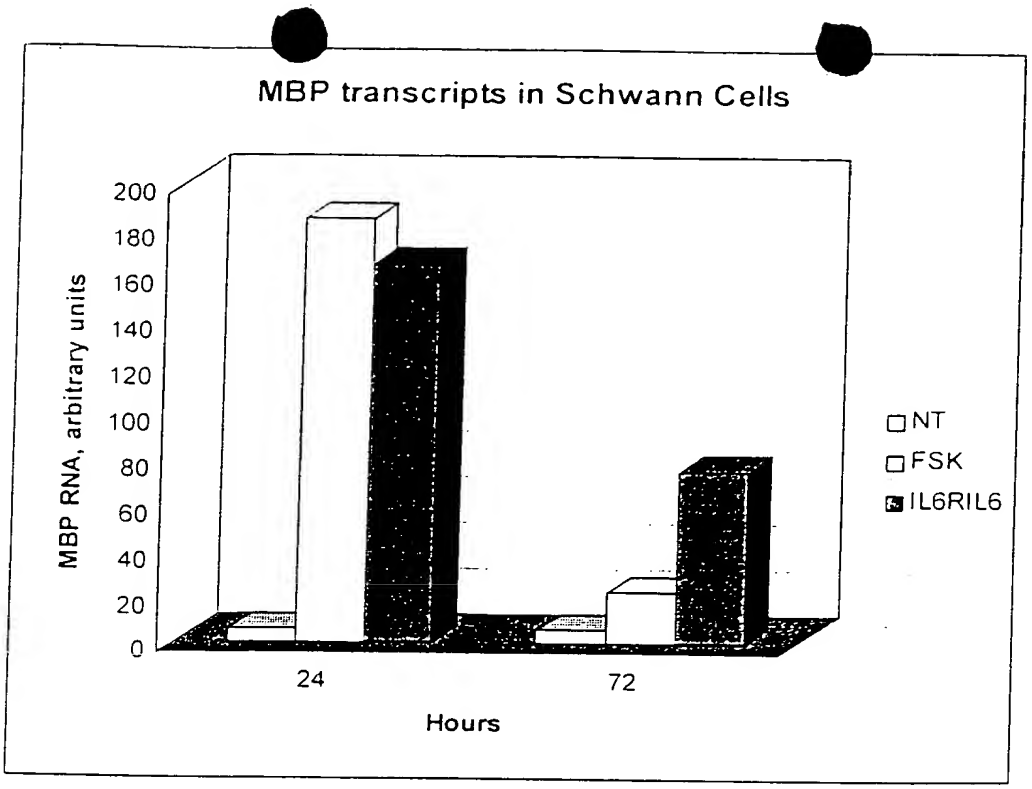
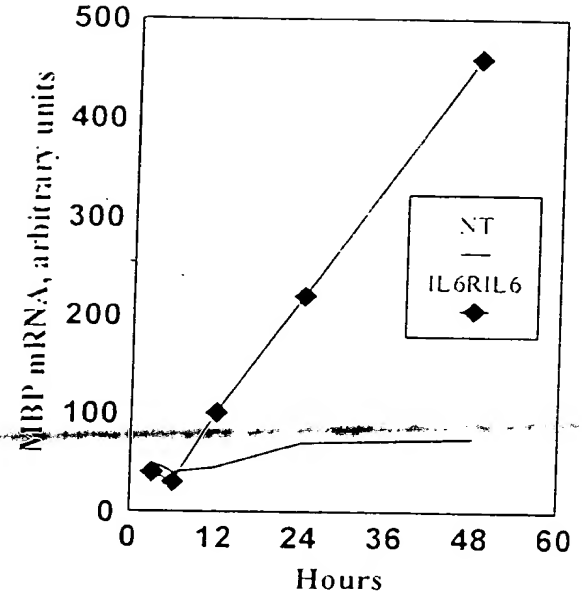

MBP transcripts in Schwann Cells
MBP RNA, arbitrary units
200
180
160
140
120
100
80
60
40
20
0
24
72
Hours
NT
FSK
IL6RIL6

FIGURE 1

A


500
400
300
200
100
0
MBP mRNA, arbitrary units
NT
IL6RIL6
0
12
24
36
48
60
Hours

B

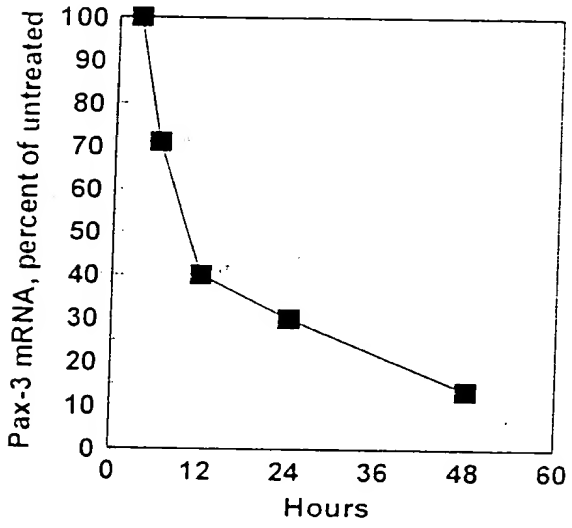

100
90
80
70
60
50
40
30
20
10
0
Pax-3 mRNA, percent of untreated
0
12
24
36
48
60
Hours

FIGURE 2